LARVAL DEVELOPMENT OF BALANUS EBURNEUS IN THE LABORATORY [1]

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Most of the descriptions of larval development of barnacles have been based upon material obtained from the plankton. By this method Willemoes-Suhm (1876) found that *Lepas fascicularis* passed through six free-swimming naupliar stages and one cyprid stage. Since his publication others have made similar studies on different species of acorn barnacles and reported that they may pass through from six to eight naupliar stages and one cyprid stage. Because of the similarity of naupliar and cyprid stages of different species in the plankton the sequence of stages in reconstructions has been questioned. Hence many investigators have attempted to verify reconstructed life cycles by rearing barnacles from the egg to the sessile stage in the laboratory.

Attempts to rear nauplii from unhatched eggs or from naupliar stages in the plankton have met with limited success. Groom (1894a), for example, only maintained *Balanus perforatus* nauplii through the second stage and Treat (1937) was unable to rear *Balanus balanoides* beyond the third stage. Sandison (1954) was also unsuccessful in maintaining several South African barnacles (*Balanus algicola, Balanus amphitrite denticulata, Balanus maxillaris, Balanus trigonus, Chthamalus dentatus, Octomeris angulosa* and *Tetraclita serrata*) beyond the third naupliar stage. Bassindale (1936) was able to rear *Verruca stroemia* to the cyprid stage by feeding the nauplii on *Nitzschia* sp. but the cyprids failed to settle. By using the same methods he could raise *Balanus balanoides* to the fifth naupliar stage only. Batham (1945) maintained the non-feeding nauplii of the goose-barnacle, *Pollicipes spinosus,* from the egg to a stage described as "post-cypris," but no attachment occurred. There have been two definite reports of barnacles being successfully reared from the egg to the settled pin-head: that of Herz (1933) for *Balanus crenatus* and Hudinaga and Kasahara (1941) for *Balanus amphitrite hawaiiensis.* Hudinaga and Kasahara also reared *Tetraclita squamosa* on *Skeletonema costatum* and *Nitzschia closterium* but they all died in the cyprid stage. Pyefinch (1948b, 1949a) refers to culturing and describes the larval stages of *Balanus crenatus,* giving the time of appearance after hatching for the individual stages in the laboratory. He does not discuss the duration of the stages or mention successful attachment and metamorphosis of the cyprid. All investigators to date have reared larvae in mass culture and have determined time and stage of molting by daily sampling. While this method may indicate the number of larval stages it does not give accurately the molting frequency or the variations in intermolt periods within the population nor does it take into account the per cent mortality.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Duke University NR 163-194.

The barnacle selected for this study was *Balanus eburneus,* for neither has it been reared in the laboratory nor have its larval stages been described, even though it has a range from Massachusetts to South America (Pilsbury, 1916). Its larval history is known only through the report of Grave (1933) who merely stated that it passes through naupliar, metanaupliar, and cyprid stages in 7 to 10 days at Woods Hole, Mass. Therefore, the present study was undertaken to determine the food which would support complete development, the number and description of the larval stages, the frequency of molting, duration and mortality of each intermolt and the length of time required for complete development.

Methods

Intact adult *Balanus eburneus* were removed from pilings and cleaned of attached organisms. In the laboratory the basis of the barnacle was chipped away and the egg lamellae removed. The developing ova obtained and successfully reared had attained the distinct median eye spot and gray color which corresponds to the "H" stage of Groom (1894a). The lamellae were placed in finger bowls containing filtered sea water with a salinity of 28.5 per thousand, *Chlamydomonas* sp., and 200,000 to 400,000 units of penicillin per liter. The bowls were then covered and maintained at 26° C., the average outside water temperature, in a constant-temperature culture cabinet lighted by daylight fluorescent lamps. In order to obtain nauplii of known age only those which were observed to hatch were used. At the time of removal each nauplius was placed in a separate compartment of the rearing assembly. The assembly was made by drilling 100 holes in a piece of ⅜″ lucite with a second solid piece of lucite forming the bottom. Each well had a capacity of 1.2 cc. The assembly was then placed in a glass dish, covered, and maintained in the culture cabinet. The contents of the wells were checked two to three times daily with a binocular dissecting microscope. When an exuvium was found it was removed, placed in 70 per cent alcohol, and the time, number of the molt, and mortality recorded for each nauplius. After the second molt freshly fertilized *Arbacia punctulata* eggs were introduced daily into the compartments in addition to the *Chlamydomonas* sp. At this time the larval plutei, developed from the eggs of the previous day, were removed.

The naupliar stages were determined from the number of molts under segregated conditions. These were drawn to scale on graph paper, with the aid of a Whipple disc, from the exuviae, fixed specimens, and, in a few cases, from the living nauplii. The setation formulae were obtained from the exuviae and dissected appendages of known stages. Measurements were made with an ocular micrometer mounted in a compound microscope. In addition to the 121 nauplii raised in individual compartments, hundreds of newly hatched larvae were maintained in finger bowls and plastic compartmented boxes. From these sources specimens were fixed daily in 70 per cent alcohol and 60°-C. Bouin's. After the stages had been determined from exuviae of segregated barnacles the fixed specimens were staged and studied to determine the consistency in appendage setation.

Results and Discussion

The nauplii of *Balanus eburneus,* reared individually, pass through six stages and one cyprid stage, a conclusion which is consistent with Bassindale's (1936) rear-

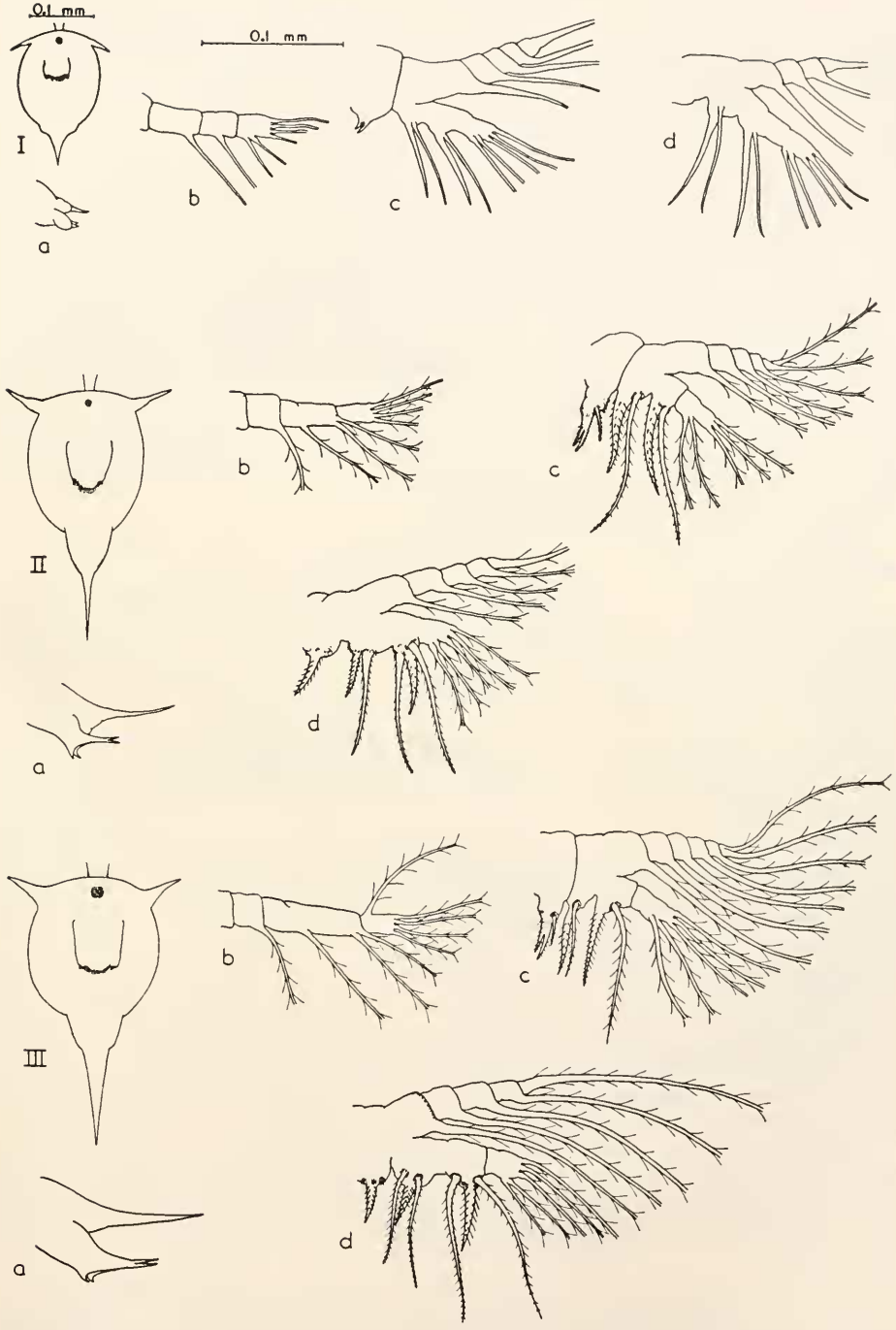

FIGURE 1. Carapace, caudal and abdominal processes, and appendages of naupliar stages I, II, and III of *Balanus eburneus* reared in the laboratory. All swimming setae are cut short. a, lateral view of abdominal and caudal processes; b, antennule; c, antenna; d, mandible.

ing of *Verruca stroemia,* and *Chthamalus stellatus* and the reconstructions of larval stages obtained from the plankton on *Balanus perforatus* (Groom, 1894b), *Balanus balanoides, Balanus crenatus, Verruca stroemia* (Pyefinch, 1948a), *Elminius modestus, Balanus improvisus, Balanus crenatus* (Knight-Jones and Waugh, 1949), and *Balanus algicola, Balanus trigonus, Octomeris angulosa* (Sandison, 1954). However, our results, Bassindale's (1936), and those based on reconstructions from the plankton do not agree with those obtained from culture methods reported by Herz (1933) and Hudinaga and Kasahara (1941). Herz (1933) found that *Balanus crenatus* passed through eight naupliar stages and one cyprid stage and Hudinaga and Kasahara (1941) reported seven naupliar stages and one cyprid stage for *Balanus amphitrite hawaiiensis.* The discrepancy between staged barnacle larvae obtained from the plankton and those taken from mass culture by Herz (1933) and Hudinaga and Kasahara (1941) may be due to the use of appendage setation and other morphological characteristics as the main criteria for staging rather than the use of the exact number of observed naupliar exuviae from the egg to the cyprid stage. As is shown below, setation and spine structure were considered in this study but the number of naupliar stages was based solely on the exact number of molts through which each individual passed.

Nauplii. The most significant characters for each naupliar stage are given below.

Stage I. (Fig. 1, I.) The small frontolateral horns appear slightly recessed and project caudally. The horns are frequently hidden on the ventral side by the antennules and antennae. The caudal process is short and blunt and the abdominal process terminates in two short spines (Fig. 1, Ia). All setae are devoid of setules.

Stage II. (Fig. 1, II.) The frontolateral horns project from the carapace at approximately right angles. The bases of the horns appear slightly swollen. The abdominal process now bears one spine on each side of the base and is approximately half the length of the caudal process (Fig. 1, IIa). All setae bear setules.

Stage III. (Fig. 1, III.) The frontolateral horns are tapered gradually from the junction with the carapace. The abdominal process bears the same spines as in stage II but is now greater than half the length of the caudal process. While larger and slightly heavier than stage II the primary distinguishing features are differences in setation of the antennules and antennae.

Stage IV. (Fig. 2, IV.) The posterior edge of the carapace is delimited from the caudal process for the first time and bears a pair of carapace spines. The abdominal process bears two spines on each side. One pair is located at the base and the second pair in line with the division between the caudal process and the abdominal process (Fig. 2, IVa).

Stage V. (Fig. 2, V.) There are two pairs of spines near the terminal portion of the abdominal process, a large lateral pair and a smaller median pair. Anterior to these is a pair of spines between which is a small, mid-ventral spine. The base is quite swollen and shows partial segmentation. The maxillule first appear (Fig. 2, Va).

Stage VI. (Fig. 2, VI.) The abdominal process is considerably enlarged over the fifth stage and six pairs of small spines mark the developing cirriform appendages beneath the exoskeleton. The spines on each side of the mid-ventral

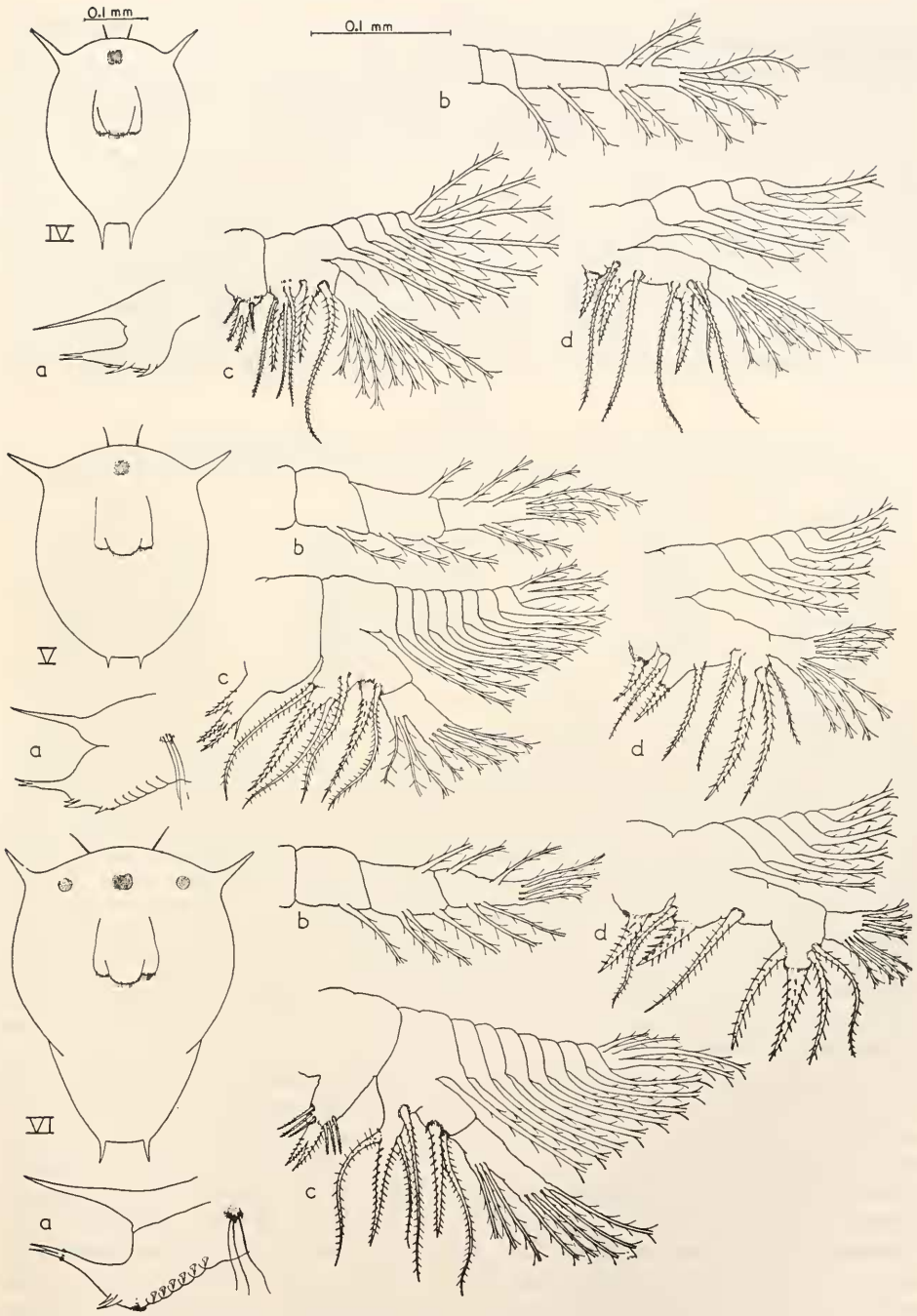

FIGURE 2. Carapace, caudal and abdominal processes, and appendages of naupliar stages IV, V, and VI of *Balanus eburneus* reared in the laboratory. All swimming setae are cut short. a, lateral view of abdominal and caudal processes; b, antennule; c, antenna; d, mandible.

spine, found in the fifth stage, are no longer present. The paired eyespots become quite distinct in the later sixth stage nauplii.

Cyprid. No significant characteristics were observed which distinguish *Balanus eburneus* cyprids from those of *Balanus amphitrite denticulata* (unpublished data).

Table I gives the setation formulae for each naupliar stage of *Balanus eburneus.* Since Bassindale's (1936) introduction of this system it has been applied to nauplii of many species of barnacles. Unfortunately, as asserted by Bassindale, the setation formulae alone do not give a definite indication of stage or species. Knight-Jones and Waugh (1949) point out the extreme differences between types of setae and believe it to be misleading. They note a remarkable similarity between the setation of earlier stages of several species. Stage I of *Elminius modestus, Balanus improvisus* and *Balanus crenatus* were found to be identical and stage II of *Elminius modestus, Chthalamus stellatus,* and *Verruca stroemia* had corresponding setation. San-

TABLE I

Setation formulae of the six naupliar stages of Balanus eburneus

Stage	Antennule	Antenna	Mandible
I	0.4.2.1.1.	0.1.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.2.2.G.
II	0.4.2.1.1.	0.2.4.–0.3.2.2.2.G.	0.1.4.–0.3.2.3.2.G.
III	1.4.2.1.1.	0.2.5.–0.3.2.2.2.G.	0.1.4.–0.3.2.3.2.G.
IV	1.1.4.2.1.1.	0.3.5.–0.5.3.2.4.G.	0.1.4.–0.4.2.4.3.G.
V	1.1.1.4.2.1.1.1.	0.3.8.–0.5.3.2.4.G.	0.1.5.–0.4.4.4.3.G.
VI	1.1.1.4.2.1.2.1.	0.4.8.–0.5.3.2.4.G.	0.1.5.–0.4.4.4.3.G.

dison (1954) gives the setation formulae for *Balanus algicola, Octomeris angulosa,* and *Tetraclita serrata.* The first stage of *B. eburneus* corresponds in setation to those forms listed by Sandison (1954) and also *B. crenatus* which in turn makes the first stage of *B. eburneus* identical to *E. modestus* and *B. improvisus.* The setation of the second to sixth stages of *B. eburneus,* to our knowledge, is different from that described for any other species. Norris, Jones, Lovegrove and Crisp (1951) found the setation formulae to be of very limited value in studies on *Balanus perforatus, B. improvisus,* and *B. amphitrite denticulata* and suggest setation to be a developmental feature rather than a specific one for the separation of barnacle larvae. In both *B. improvisus* and *B. amphitrite denticulata* Norris *et al.* (1951) found some variation in the setation of the mandibular exopodite of stage II. They observed that it may "sometimes" resemble the third stage by bearing five setae instead of four and suggest it as a possible explanation for the eight stages found by Herz (1933). Pyefinch (1949a), however, examined thousands of staged nauplii of *Balanus crenatus* and found setation to be uniform within each stage. Norris *et al.* (1951) propose that the rate of morphological development of eyes, limbs, and setae is more strongly influenced by temperature than the rate of growth and onset of ecdysis and consequently the latter do not always occur in step with the former. Hudinaga and Kasahara (1941) note that there is no change in setation of the three appendages between the sixth and seventh stages of *B. amphitrite hawaiiensis.* They discriminate between the two stages primarily on the presence of completely developed paired eyes in the seventh stage as compared to the rudimentary nature in the sixth stage. The present study of *B. eburneus,* in addition to the accounts of other species

by Pyefinch (1949a), Bassindale (1936), and Sandison (1954), shows that the change from rudimentary to completely developed paired eyes takes place in the sixth stage. The development of *B. eburneus*, under controlled temperature conditions, does not reveal any variation in setation within any one stage. Exuviae collected from nauplii undergoing a known molt showed consistency in the setation for that particular stage.

Pyefinch (1949a) describes the third stage of *B. crenatus* as the first one with a delimited carapace bearing a pair of carapace spines. This feature is seen first in the fourth stage of *B. eburneus* and also in *Balanus algicola, Balanus trigonus, Octomeris angulosa* (Sandison, 1954) and *Pollicipes spinosus* (Batham, 1945). The consistency of the mandibular exopodite setation in all 6 naupliar stages of *B. crenatus* appears to be an unusual feature when compared with the increase in setation described for other species.

TABLE II

Measurements of larval stages of Balanus eburneus reared under laboratory conditions

Stage	Carapace		Total length (mm.)
	Width (mm.)	Length (mm.)	
I	0.16–0.18	—	0.19–0.23
II	0.21–0.23	—	0.32–0.34
III	0.23–0.27	—	0.35–0.38
IV	0.27–0.32	0.30–0.33	0.40–0.42
V	0.29–0.34	0.35–0.38	0.44–0.48
VI	0.36–0.39	0.42–0.50	0.54–0.60
Cyprid	—	—	0.46

Table II gives the range in size of the six naupliar stages and one cyprid stage of *B. eburneus*. Knight-Jones and Waugh (1949), using size distribution followed by setation formulae for staging planktonic material, found greater variation in size in the later stages. They also note that "total length" of nauplii is affected by flexing of the abdomen and thus this measurement may be erroneus. Greater variation in size of the later stages was also observed in this study of *B. eburneus* in the laboratory. With setation and observable internal development, such as paired eyes and cirriform appendages, the carapace width and length would be more reliable than total length for staging laboratory reared larvae and presumably planktonic material. The latter study, however, must be made before definite conclusions can be drawn.

Figure 3 gives the variation in duration of intermolt period for the individual stages. Grave (1933) postulated 7 to 10 days as the time required for over-all development of *B. eburneus* at Woods Hole but did not include periods for each stage. Hudinaga and Kasahara (1941) estimated that the individual naupliar stages of *B. amphitrite hawaiiensis* lasted one day each and Pyefinch (1948b), using plankton samples taken at three-day intervals, postulated that each stage of *Balanus balanoides* was three days, or less, in duration. In the present study the duration of the first stage of *B. eburneus* ranged from 15 minutes to 4 hours. Many authors have reported that it is quite short in other species and Hudinaga and Kasahara (1941)

noted that it lasted for 15 minutes to 2 hours for *B. amphitrite hawaiiensis*. The second stage of *B. eburneus* lasts approximately 24 hours and the third 36 hours but with greater variation (Fig. 3). Stage IV averages 2 days, stage V, 2.6 days, and stage VI, 2.5 days but with less uniformity than the earlier stages.

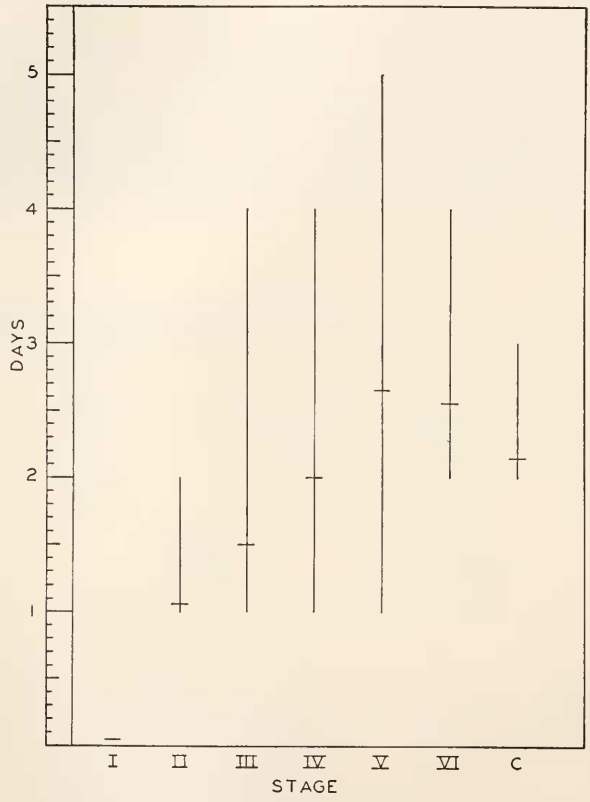

FIGURE 3. Duration of intermolt of naupliar stages (I–VI) and cyprid stage (c) of 121 *Balanus eburneus* reared under segregated conditions. The vertical line indicates the range. The horizontal line indicates the mean.

One hundred per cent of the first stage nauplii underwent ecdyses from 15 minutes to 4 hours after hatching. This was true whether food was available or not. The second stage nauplii molt from 10 to 35 hours after hatching. During this stage the gut appeared green with *Chlamydomonas* sp. The third stage nauplii may molt from the second to fifth days (Fig. 4). As indicated by Sandison (1954) this is a period when many nauplii die if proper conditions are not present. Therefore, it was at this stage that we added to the diet of *Chlamydomonas* sp. fertilized *Arbacia* eggs to furnish food of animal origin. The latter were ingested and the gut took on the echinochrome color of the *Arbacia* eggs. Even so, there was a mortality of 6 per cent (Fig. 5). The fourth naupliar stage shows a greater degree of variation in molting and ecdysis may occur from the third to eighth day with a majority molting on day 4 (Fig. 4). This is accompanied by an increase in mortality (Fig.

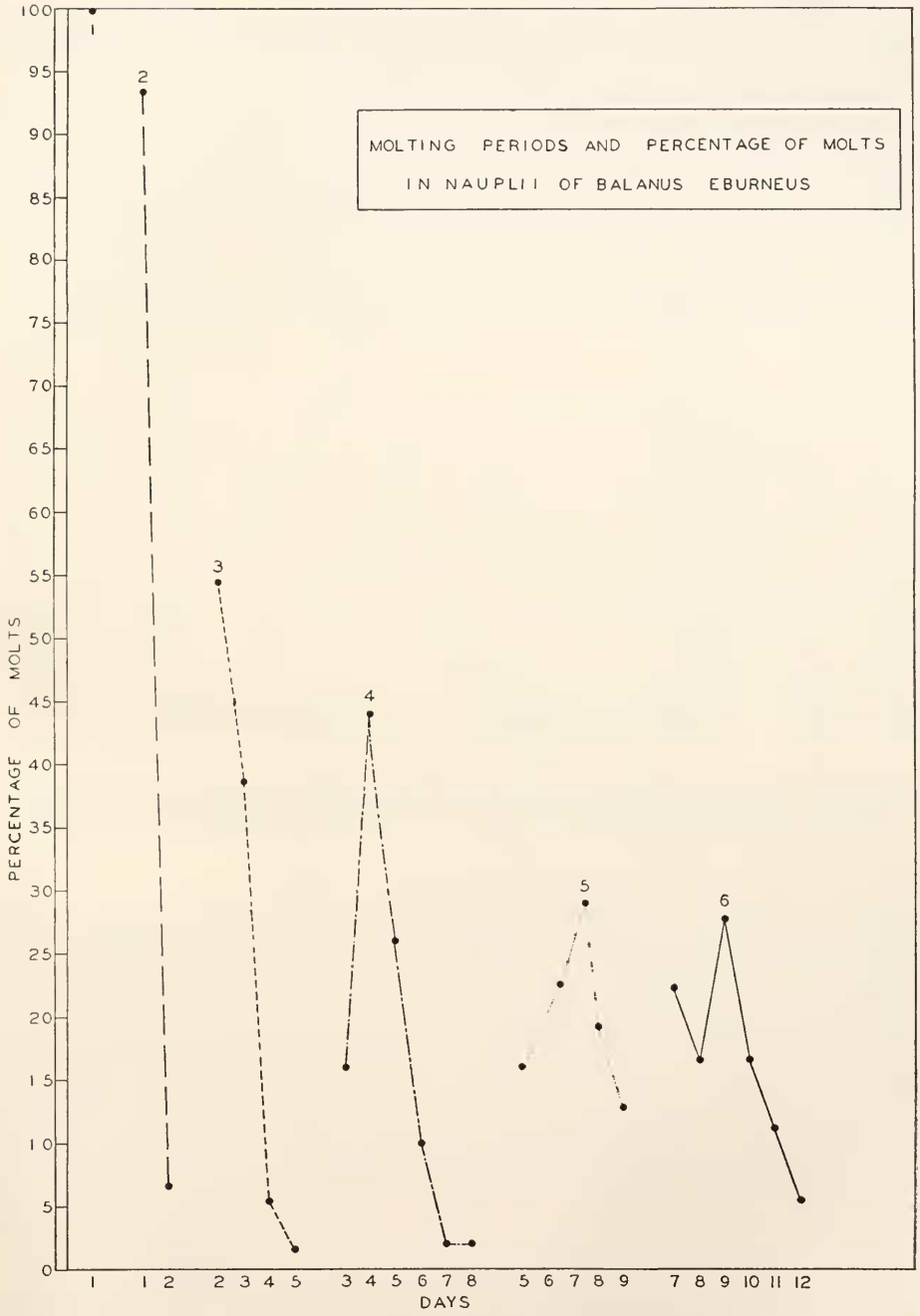

FIGURE 4. Molting frequency of naupliar stages of 121 *Balanus eburneus* reared under segregated conditions. Molts are indicated by numbers.

5). Molting of the fifth naupliar stage occurs from day 5 to 9, with a majority on day 7. This stage showed greater mortality than any other stage, it being approximately 33 per cent. The sixth and final naupliar stage molts into a cyprid on the seventh to twelfth day, with a mortality of 22 per cent.

There is apparently considerable variation in the duration of the cyprid stage. Pyefinch (1948b) reports that the cyprid of *Balanus balanoides* remains free-swimming in laboratory tanks for five days at 4–5° C. but estimated a shorter period in nature. Under laboratory conditions we found that those which settled and underwent metamorphosis to the pinhead did so within one to three days, whereas

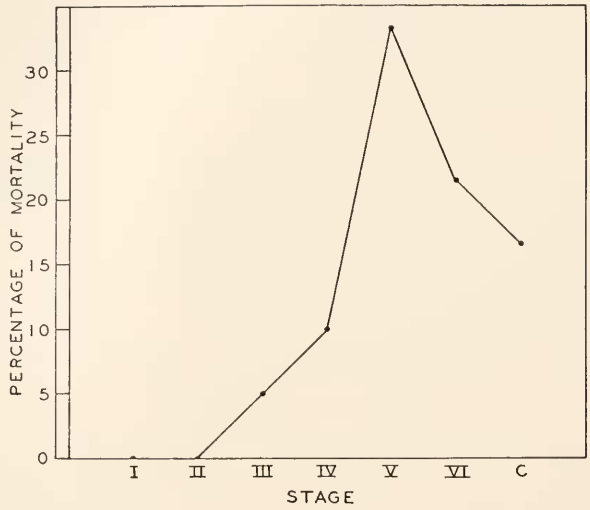

FIGURE 5. Mortality in relation to naupliar stages (I–VI) and cyprid stage (c) of 121 *Balanus eburneus* reared under segregated conditions.

those which persisted as cyprids for longer periods, up to 14 days, failed to settle and died. Mortality in the cyprid stage was approximately 16 per cent. In some cases the mortality was due to incomplete closing of the carapace following the sixth naupliar molt. These abnormal cyprids could live as swimming larvae for two to three days but they failed to settle.

The over-all time of development for *B. eburneus* is quite short when compared with the times given for most other species. Bassindale (1936), although not giving water temperatures, reported 13 days as the minimum time for completion of the sixth stage of *Chthamalus stellatus* and a 22-day minimum for the sixth stage of *Verruca stroemia*. Unfortunately, comparison between species is not too reliable, for the effect of temperature on individual species has not been determined and can only be inferred from studies on other species at different temperatures. Hudinaga and Kasahara (1941) found that the minimum time for development of *B. amphitrite hawaiiensis*, from the first naupliar stage to settling, was 7 days at 23–28° C. This falls within the range found by us for *B. eburneus* at 26° C. Grave (1933) postulated from planktonic material that *B. eburneus* takes 7–10 days for complete development. This figure corresponds with ours even though the temperature at Woods Hole is normally 4 to 8° C. lower than at Beaufort.

The best evidence to date that reduced temperatures increase the time of over-all development of barnacles is shown in the work of Pyefinch (1948b) and Batham (1945). Pyefinch (1948b) found that the over-all development of *B. crenatus* took approximately 30 days at 4–5° C. whereas at 15° C., "or more," the time was reduced to 16 days. Batham (1945) found that the goose-barnacle, *Pollicipes spinosus*, takes 12 days to pass through all larval stages at 18° C. while in the "cold room" (temperature not given) it required 20 to 21 days. The times given by Batham (1945), however, did not include normal completion of the cyprid stage or settling.

The relationships of the substratum and physical factors to settling are outside the scope of this paper. In the present study the only substratum offered was lucite and the physical factors of temperature and light were similar throughout the experiment, in that the temperature was maintained at 26° C. and the rearing assembly received constant illumination from below.

There is always the question of normality when organisms are reared in the laboratory and the query of how survival compares with that in nature. In this experiment if a barnacle completed development, settled, and metamorphosed it was considered normal. Pyefinch (1949b) estimated the survival of barnacle larvae to be between 1 and 9 per cent, depending on the species with which he was working. Bousfield (1955) questions his estimate because of the methods used and was of the opinion that natural survival was approximately 10 per cent. Therefore, our observed survival of 16.3 per cent, under laboratory conditions, is higher than that estimated in nature. Whereas it is undoubtedly true that the chief sources of mortality in nature are dispersal seaward and predation, improper food and bacteria are the chief causes in the laboratory. In preliminary experiments we found that *B. eburneus, B. amphitrite denticulata,* and *Chthamalus fragilis* larvae could not be reared beyond the third stage on a diet of *Chlamydomonas* sp. alone. When developing *Arbacia* eggs and penicillin were included, complete development occurred. The actual value of the penicillin is not known but it was observed that the tendency of nauplii to stick to surfaces of the rearing assembly was considerably reduced.

Summary and Conclusions

A technique has been devised for rearing segregated barnacle nauplii, under controlled laboratory conditions, which permits daily observations on the frequency of molting, the number of stages, and the specific characteristics of each stage. From a study of 121 segregated *Balanus eburneus,* plus hundreds in mass culture, reared on *Chlamydomonas* sp. and *Arbacia* larvae at 26° C. the following conclusions may be drawn:

1. Ecdyses provide a definitive method for staging nauplii. The larval phase of *B. eburneus* consists of six naupliar stages and one cyprid stage. Secondary criteria, such as body size, spine structure, and appendage setation, are given for the larval stages.

2. The duration of the six naupliar stages is as follows: first stage, 15 minutes to 4 hours; second stage, one to two days with an average of one day; third stage, one to four days with an average of 1.5 days; fourth stage, one to four days with an average of two days; fifth stage, one to five days with an average of 2.6 days; and the sixth stage, two to four days with an average of 2.5 days.

3. The cyprid stage ranges from one to fourteen days but successful attachment was observed only in those which settled one to three days following the final naupliar molt.

4. The over-all larval development in the laboratory ranges from 7 to 13 days.

5. The first ecdysis occurs from 15 minutes to 4 hours after hatching and is usually followed by the second molt during the first day. The third molt occurs from the second to the fifth days and the fourth molt takes place from the third to the eighth day. The fifth ecdysis occurs from the fifth to the ninth day with the sixth molt ranging from the seventh to the twelfth day.

6. Tables are given for the size of the nauplii and the setation of the appendages.

7. Successful metamorphosis and attachment was observed in 16.3 per cent of the 121 barnacle nauplii studied under segregated laboratory conditions.

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